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GONOSPORA HOLOFLORA: A NEW SPECIES OF GREGARINE PROTOZOAN PARASITE (APICOMPLEXA) IN *HOLOTHURIA FLORIDANA* (ECHINODERMATA: HOLOTHUROIDEA) FROM THE FLORIDA KEYS

Christopher M. Pomory and Michael T. Lares

ABSTRACT

Four of 50 sea cucumbers, *Holothuria floridana,* from the Florida Keys were infected with a new species of gregarine protozoan parasite. *Gonospora holoflora* n.sp. has pyriform gamonts up to 400 μm long, slightly ovoid gametocysts 125 μm by 150 μm, and oval oocysts 7 μm by 11 μm without tails. The parasite remains attached to the coelom-side of the holothuroid intestine at least until the terminal stage cyst is reached. The holothuroid exhibited a coelomocyte reaction to the parasitic infection producing numerous lymphocytes that covered the coelom-side of the intestine.

Symbiotic relationships, used in the broad sense of commensalism, parasitism, and mutualism (Saffo, 1993), have long fascinated biologists. Echinoderms harbor a number of other taxa including protozoans, crustaceans, annelids, and fish (Jangoux, 1987a, 1987b), with many associations reported for the Caribbean fauna (Hendler et al., 1995). Gregarines (phylum Apicomplexa) are parasites of a variety of invertebrates, including holothuroids and echinoids, usually inhabiting the coelom or intestine (Levine, 1985, 1988). None has been described from the echinoderm fauna of the Caribbean (Jangoux, 1987a).

The gregarine life-cycle includes a series of stages nested within one another (Levine, 1971, 1988). The sporozoite is the infecting stage. After ingestion by the host it develops into a trophozoite that attaches to the intestine and grows into the coelom. The trophozoites are haploid and become gamonts that undergo cytoplasmic fusion. The two nuclei from the gamonts divide forming gametes within a gametocyst. The gametes unite forming zygotes which grow into oocysts containing eight haploid sporozoites (old trophozoite containing gametocyst containing oocysts containing sporozoites). When the sporozoites are released from the body the cycle repeats. Cysts at various stages are found attached to the host at the same time. Most gregarines are thought to be host specific or confined to closely related species in the same locality, although the host may be infected by more than one species (Levine, 1977, 1979, 1985, 1988).

The sea cucumber *Holothuria floridana* Pourtalès is a common holothuroid of the Caribbean region. It is found in shallow-water (<2 m) with adults primarily in seagrass beds and younger individuals underneath rock rubble (Hendler et al., 1995). This paper describes a new species of gregarine parasite from *H. floridana,* and the coelomocyte reaction of the host.

MATERIALS AND METHODS

Fifty *Holothuria floridana* were collected from underneath rocks at Missouri Key, Florida (24°40.5'N, 81°14.9'W) in March, 1994. They were kept in a laboratory holding tank (35‰ salinity and 22°C) for 1 mo. Upon dissection of the holothuroids an undescribed species of gregarine was encountered that was visible to the unaided eye in the most highly infected individual. The holding tank was cleaned and dried before use, only contained the holothuroids, and did not contain bottom sediment or other debris. Therefore, the tank was not the source of the parasites.

A portion of the holothuroid intestine containing the parasites and a portion of intestine from four apparently non-parasitized individuals were preserved in Trump's fixative, formaldehyde/glutaralde-

hyde in phosphate buffer (McDowell and Trump, 1976), for SEM. After fixing, samples were rinsed in phosphate buffer, dehydrated through an alcohol series, and critical point dried using CO_2 as the transition fluid. They were then mounted on stubs and sputter coated with gold-palladium for 100s. The stubs were viewed using a JEOL JSM 35 scanning electron microscope with an accelerating voltage of 15 kV, and a working distance of 15 mm. Negatives were made with a Polaroid attachment. Fresh tissue was also observed under a dissecting scope, and photomicrographs were made through an Olympus Vanox compound microscope with a C-35a camera mount. Description of the new species follows the terminology of Levine (1971, 1988).

RESULTS

Higher taxonomy after Levine (1988):

Phylum Apicomplexa Levine, 1970
Class Conoidasida Levine, 1988
Subclass Gregarinasina Dufour, 1828
Order Eugregarinoidia Léger, 1900
Suborder Aseptatorina Chakravarty, 1960
Family Urosporidae Léger, 1892
Genus *Gonospora* Schneider, 1875
Gonospora holoflora new species
Figures 1 and 2

Diagnosis.—Gamonts (trophozoites) large, pyriform-shaped, up to 400 μm long. Oocysts oval, 7 μm by 11 μm. Trophozoites attached to the coelom-side of the intestine of *Holothuria floridana.*

Description.—GAMONTS (TROPHOZOITES): large, visible to unaided eye, elongate-pyriform in shape, up to 400 μm long (Fig. 1A), white in color, young trophozoites (20 μm long) with longitudinal ridges not seen in older stages. GAMETOCYSTS: slightly oval, 125 μm by 150 μm (Fig. 1B), forming opaque ball in upper end of transparent trophozoite. OOCYSTS: oval, 7 μm by 11 μm (Fig. 1C,D), one end rounded, one end funnel-shaped, without long tails or processes, oocysts completely fill trophozoite turning it translucent (Fig. 1C). All stages through oocyst formation attached to intestine (Fig. 2A,B). TYPE HOST: *Holothuria floridana* Pourtalès. INFECTION SITE: attached to the coelom-side of the beginning and middle of the intestine, not seen at the beginning and end of the complete digestive track. TYPE LOCALITY: Atlantic side of Missouri Key, Florida (24°40.5′N, 81°14.9′W), shallow-water (<1 m), a mosaic of limestone hard-pan (attached sponges and soft corals), calcium carbonate sand patches, scattered rocks, and seagrass beds.

Etymology.—Combination of the genus and species name of the host *Holothuria floridana.*

Remarks.—Only one individual was infected to the point of the trophozoites being visible to the unaided eye. The density of the infection was similar to Figure 2A (8 mm^{-2}) for an 8 cm section of intestine. Upon examination of the SEM material, three more holothuroids were found to have early stage infections.

The parasitic infection produced a large coelomocyte reaction (Figure 2B,C) that was not seen in an uninfected individual (Fig. 2D). The coelomocytes (lymphocytes) covered the surface of the intestine forming an unbroken "carpet" in the infected region, but were not found in great numbers attached to the trophozoites.

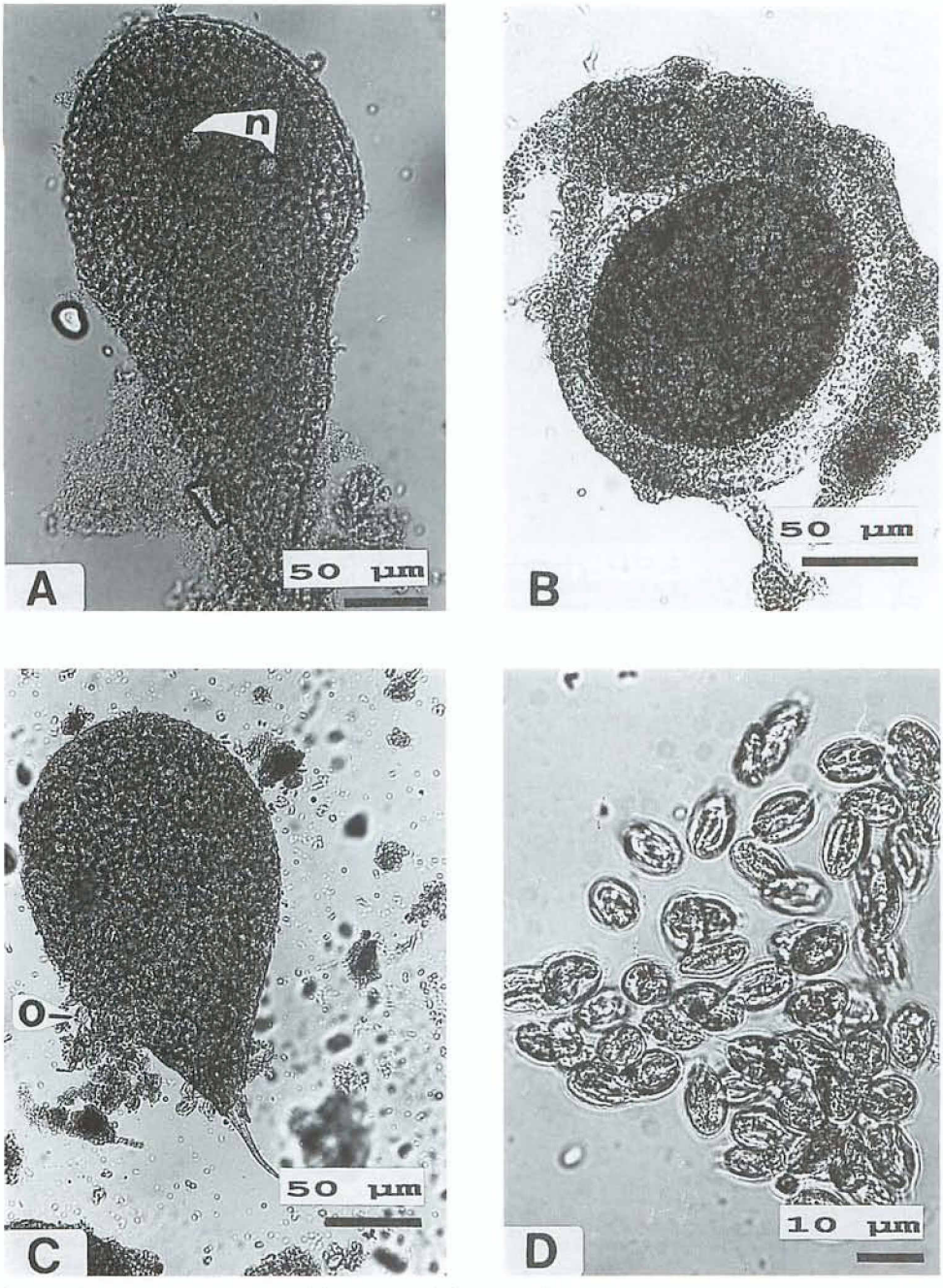

Figure 1. Light micrographs of the stages of the gregarine *Gonospora holoflora* n.sp. A: Gamonts after syzygy (n = two nuclei). B: Gametocyst before oocyst development. C: Gametocyst after oocyst development (o = oocysts spilling out of small tear). D: Oocysts containing sporozoites.

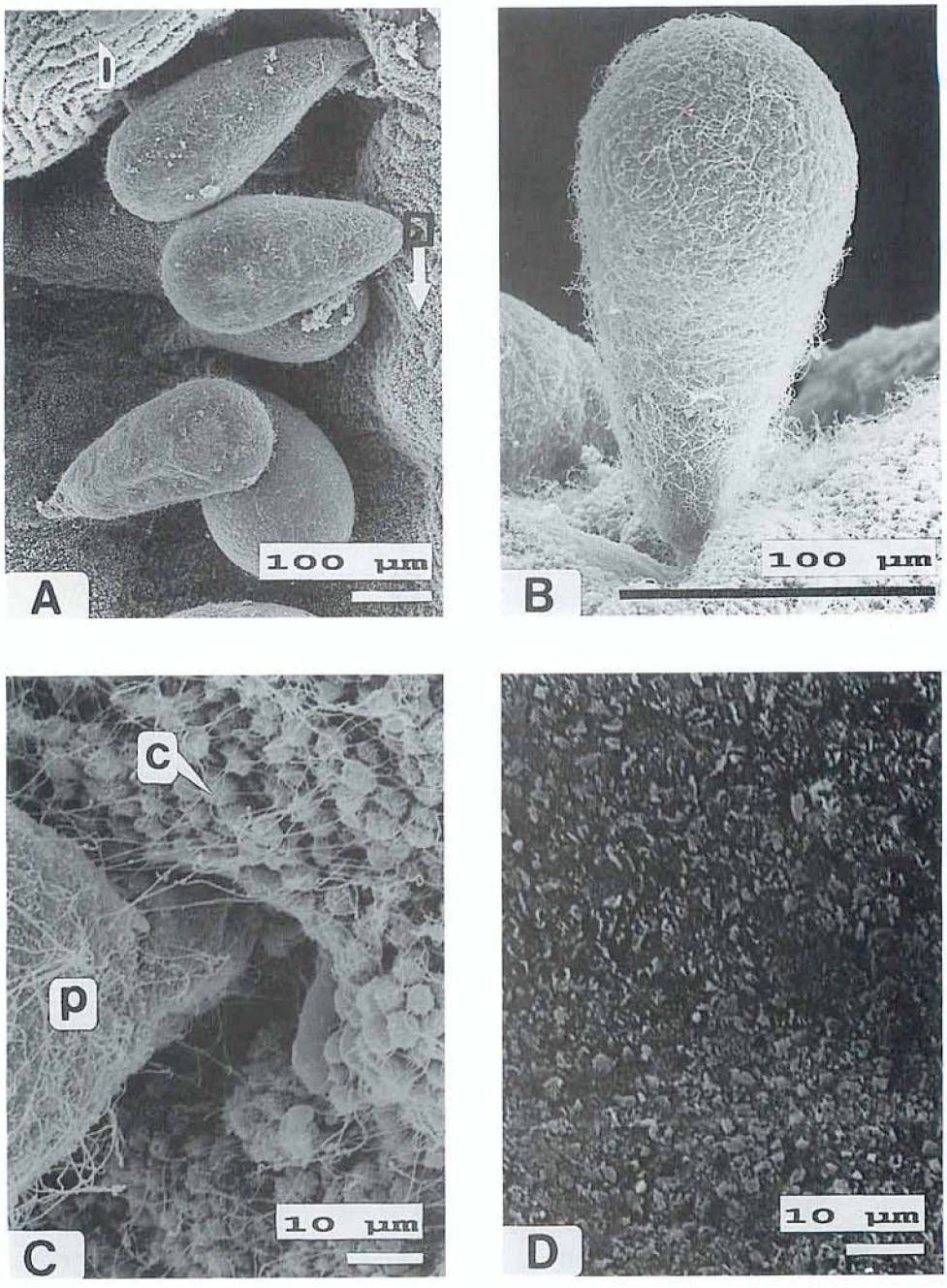

Figure 2. Scanning electron micrographs of advanced stages of cyst formation of the gregarine *Gonospora holoflora* n.sp. on the intestine of the holothuroid *Holothuria floridana*. A: Coelom-side of the intestine of *H. floridana* with various stage trophozoites (gamonts, gametocyst, oocyst) showing density of infection (i = intestinal surface; area within rectangular box is enlarged in part C). B: Terminal stage trophozoite (after oocyst formation). C: Closeup of parasite attachment and coelomocyte "carpet" (p = parasite; c = coelomocytes of holothuroid). D: Intestinal surface from a non-parasitized *H. floridana* showing a complete lack of a coelomocyte reaction (C & D are at same magnification, 900×).

Discussion

The gregarine family Urosporidae contains three genera (*Lithocystis, Urospora, Gonospora*) that infect echinoderms (Levine, 1977, 1988; Jangoux, 1987a). The three genera are very similar and at one time were lumped together in the genus *Cystobia* (Jangoux, 1987a; Levine, 1988). Whether they should be separate genera or one genus is still in debate as species often have characteristics of all three genera (Levine, 1977, 1985, 1988), including the new species. In the new species, the general shape and size of the gamonts are similar to *Urospora,* and the long time of attachment to the intestine is similar to *Lithocystis* (Levine, 1985, 1988). The main distinguishing characteristic is the oocyst (Levine, 1977) which fits the description of *Gonospora* (Levine, 1985, 1988). The other two genera have tail-like extensions on the oocyst that the new species lacks (e.g., illustrated in Coulon and Jangoux, 1987). This is the first gregarine to be described from a Caribbean holothuroid. Most descriptions are from European waters (Levine, 1977, 1985, 1988; Jangoux, 1987a).

The holothuroid exhibited a coelomocyte reaction which is probably an immunological response to the parasitic infection (Endean, 1966; Jangoux, 1987a; Smiley, 1994). The coelomocytes were all similar, fitting Hetzel's (1963) subcategory of lymphocytes, having a spheroid cell body with thread-like projections. The lymphocytes formed a continuous layer or "carpet" over the surface of the intestine, presumably to prevent attachment of parasites. A coelomocyte reaction has been described for the echinoid *Echinocardium cordatum* that was infected with sporozoan parasites (De Ridder and Jangoux, 1985; Coulon and Jangoux, 1987). In the echinoid, the coelomocytes attached directly to the parasites leading to the death of the parasites (Coulon and Jangoux, 1987). This reaction was not seen in the holothuroid. Based on the number and different stages of cysts attached to the intestine in our specimen the immune response does not seem to stop the infection. No studies exist as to whether or not gregarine infections are deleterious to holothuroids.

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ADDRESSES: (C.M.P.) *Department of Biology, University of South Florida, Tampa, Florida 33620;* (M.T.L.) *Department of Environmental Science, St. Leo College, St. Leo, Florida 33574.*